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(21) International Application Number: PCT/US97/09895 (22) International Filing Date: 6 June 1997 (06.06.97) (30) Priority Data: 08/660,562 7 June 1996 (07.06.96) US (71) Applicant: AMGEN, INC. [US/US]; Amgen Center, 1840 De Havilland Drive, Thousand Oaks, CA 91320-1789 (US). (72) Inventors: JOHNSON, Merrie, J.; 2801 Rustic Glen Drive, Thousand Oaks, CA 91362 (US). SIMONET, William, S.; 3801 Calle Linda Vista, Newbury Park, CA 91320 (US). DANILENKO, Dimitry, M.; 13082 Vista Court, Camarillo, CA 93012 (US). (74) Agents: ODRE, Steven, M. et al.; Amgen, Inc., Amgen Center, 1840 De Havilland Drive, Thousand Oaks, CA 91320-1789 (US).		(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published <i>Without international search report and to be republished upon receipt of that report.</i>
(54) Title: TUMOR NECROSIS FACTOR-RELATED POLYPEPTIDE (57) Abstract A novel member of the tumor necrosis factor (TNF) family was identified and observed to be involved in inflammation and necrosis, especially of the liver, myelopoiesis and bone resorption. The polypeptide is termed AGP-1. Nucleic acid sequences, vectors and host cells for the expression of AGP-1 are disclosed. Methods for identifying antagonists of AGP-1, pharmaceutical compositions comprising AGP-1 and methods of treatment using AGP-1 and AGP-1 antagonists are also disclosed.		

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TUMOR NECROSIS FACTOR-RELATED POLYPEPTIDE

Field of the Invention

5 The invention relates to AGP-1, a tumor
necrosis factor-related polypeptide involved in
inflammation, myelopoiesis and bone resorption. Nucleic
acid sequences, vectors and host cells for the
expression of AGP-1 are disclosed. Also encompassed are
10 pharmaceutical compositions comprising AGP-1, methods of
identifying antagonists of AGP-1 and methods of
treatment using AGP-1 or AGP-1 antagonists.

15 Background of the Invention

 The tumor necrosis factor family is a growing
group of cytokines which function as mediators of immune
regulation, acute and chronic inflammatory responses,
and programmed cell death. Tumor necrosis factor (TNF α)
20 is the prototypical member of this family which also
includes lymphotoxin (LT α , TNF β), lymphotoxin β (LT β),
and ligands for CD27, CD30, CD40, OX40, 4-1BB, and Fas.
Homology among these family members is confined to the
carboxy-terminal 150 amino acid residues, with the
25 highest degree of homology within the β -strand regions
involved in subunit contacts which lead to
oligomerization. With the exception of LT α , which is a
secreted protein, all the ligands in this family are
type II membrane proteins. The homologous
30 carboxy-terminal domains are extracellular, and the
shorter non-homologous amino-terminal regions are
intracellular. The membrane bound form of TNF α can be
the target of proteolytic cleavage, generating a soluble
form of TNF α which circulates in certain disease states.

As systemic delivery of TNF α induces toxic shock and widespread tissue necrosis, TNF α may contribute to the morbidity and mortality associated with a variety of infectious diseases, including septic shock, autoimmune disorders and graft-versus-host disease.

The TNF family of cytokines exert their biological effects through their interactions with a family of receptors which are generally characterized as Type I membrane proteins with cysteine-rich pseudorepeats in their extracellular domains. Of the twelve TNF receptor superfamily members identified to date, only the two poxvirus genes, T2 and A53R, encode soluble, secreted receptors. Whereas soluble forms of TNF α play an important role in the immune response, the interaction of membrane bound ligands and receptors of this family, particularly on T and B cells, likely plays a major role in cell-cell cross-talk within the immune system. In this regard, signaling through FasL and its receptor is believed to play an important role in T-cell mediated cytotoxicity.

Perhaps the most intriguing activity associated with this family is their ability to induce programmed cell death through the apoptotic pathway, a phenomena which is crucial in many areas of vertebrate development, including T-cell development. Of the known TNF family members, TNF α , LT α and FasL have all been demonstrated to induce apoptosis of certain cells under the correct conditions. Although the apoptotic effects of TNF α and LT α appear to be limited to a minimal number of cell types, signalling through Fas has been demonstrated to induce apoptosis of numerous transformed cell lines and chronically activated T cell clones. Additionally, two mutations that accelerate autoimmune disease (*lpr* and *gld*), resulting in lymphadenopathy and splenomegaly in mutant mice, are known to correspond to

mutations within the genes encoding Fas and FasL, respectively.

In view of the involvement of TNF and TNF-related family members in conditions associated with inflammation, infectious disease, immune system disorders and apoptotic cell death, it is desirable to identify additional related TNF family members.

It is an object of this invention to identify TNF-related molecules for the purpose of developing treatments for disorders related to TNF and TNF-related molecules.

A novel gene has been identified which encodes a polypeptide having significant homology to the TNF family member FasL. The polypeptide has been termed AGP-1. Transgenic mice expressing murine AGP-1 in the liver exhibit hepatic inflammation and necrosis, bile duct hyperplasia, as well as pathological findings supportive of direct or indirect systemic effects of the factor. The nucleotide and amino acid sequence of AGP-1 was found to be identical to the sequence reported for TNF-related apoptosis-inducing ligand (TRAIL, see Wiley et al. Immunity 3, 673-682 (1995)). TRAIL was observed to induce apoptosis in a wide variety of transformed cell lines.

Summary of the Invention

A novel member of the tumor necrosis factor family, termed AGP-1, has been identified from a murine cDNA library and expressed in a transgenic mouse system. AGP-1 is involved in myelopoiesis accompanied by an increase in neutrophils and lymphocytes, inflammation and necrosis of the liver, and bone resorption. Human AGP-1 has also been identified.

The invention provides for nucleic acids encoding a polypeptide having at least one of the

biological activities of AGP-1, vectors and host cells expressing the polypeptide, and method for producing recombinant AGP-1. Antibodies or fragments thereof which specifically bind AGP-1 are also provided.

5 Methods of identifying antagonists of AGP-1 which reduce or eliminate at least one of the biological activities of AGP-1 are also encompassed by the invention. Such antagonists include peptides, proteins, carbohydrates or small molecular weight organic
10 molecules which bind to AGP-1 or to its receptor(s) and interfere with AGP-1 receptor activation.

AGP-1 may be used to treat hematopoietic disorders characterized by a decrease in cell population of the bone marrow. AGP-1 antagonists may be used to
15 treat inflammatory conditions. AGP-1 antagonists may also be used to treat bone disorders resulting from an increase in bone resorption. Pharmaceutical compositions comprising AGP-1 and AGP-1 antagonists are also encompassed by the invention.

20

Description of the Figures

Figure 1. cDNA and amino acid sequence of murine AGP-1.

25

Figure 2. cDNA and amino acid sequence of human AGP-1.

Figure 3. Hematoxylin and Eosin (H&E) stained
30 sections of liver from non-transgenic mouse #12 (A) and HEAGP F1 transgenic mouse #75-13 (B). B illustrates marked proliferative cholangiohepatitis characterized by periportal bile duct hyperplasia and inflammation (arrowheads in B; arrowhead in A points to a normal
35 portal tract for contrast) with scattered foci of hepatocellular necrosis (asterisk in A). Bars = 50 μ m.

Figure 4. Myeloperoxidase stained sections of HEAGP F1 transgenic (B - mouse #75-13) and non-transgenic (A mouse #12) spleen. B illustrates splenomegaly in the transgenic mouse primarily caused by an expanded red pulp (asterisks) due to increased red pulp myelopoiesis (arrowheads in B illustrate aggregates of myeloperoxidase positive myeloid precursors) in the transgenic spleen as well as by white pulp lymphoid hyperplasia (arrows in B vs. A). Bars = 250 μ m.

Figure 5. TRAP stained sections of bone marrow from a non-transgenic control mouse (A - mouse #12) and an HEAGP F1 transgenic mouse (mouse #75-13) illustrating an apparent increase in the number of TRAP+ osteoclasts (arrows) lining bony trabeculae in the transgenic bone marrow (B) vs. the non-transgenic marrow (A). Bars = 25 μ m.

Detailed Description of the Invention

The invention provides for a novel member of the TNF receptor superfamily, termed AGP-1. AGP-1 refers to a polypeptide having an amino acid sequence of mammalian AGP-1 or a derivative thereof and having at least one of the biological activities of AGP-1. In preferred embodiments, AGP-1 is mouse or human AGP-1. cDNA and amino acid sequences of mouse and human AGP-1 are shown in Figures 1 and 2, respectively. The biological activities of AGP-1 include, but are not limited to, involvement in myelopoiesis, inflammation and necrosis, especially in the liver, and bone resorption.

The invention provides for isolated nucleic acids encoding polypeptides having one or more of the biological properties of AGP-1. As used herein, the

term nucleic acid represents cDNA, genomic DNA, wholly or partially synthetic DNA or RNA. The nucleic acids of the invention are selected from the group consisting of:

- a) the nucleic acids as shown in Figure 1
- 5 (SEQ ID NO: 1) or Figure 2 (SEQ ID NO: 3);
- b) nucleic acids which hybridize to the polypeptide coding regions of the nucleic acids shown in Figure 1 (SEQ ID NO: 1) or Figure 2 (SEQ ID NO: 3) and remain hybridized to the nucleic acids under high
- 10 stringency conditions; and
- c) nucleic acids which are degenerate to the nucleic acids of (a) or (b).

Nucleic acid hybridizations typically involve a multi-step process comprising a first hybridization

15 step to form nucleic acid duplexes from single strands followed by a second hybridization step carried out under more stringent conditions to selectively retain nucleic acid duplexes having a degree of homology which depends upon the stringency of hybridization during the

20 second step. The conditions of the first hybridization step are generally not crucial, provided they are not of higher stringency than the second hybridization step. Generally, the second hybridization is carried out under conditions of high stringency, wherein "high stringency"

25 conditions refers to conditions of temperature and salt which are about 12-20°C below the melting temperature (T_m) of a perfect hybrid of part or all of the complementary strands corresponding to SEQ. ID. NO: 1 or, alternatively, are about 12-20°C below the T_m of a

30 perfect hybrid of part or all of the complementary strands corresponding to SEQ. ID. NO: 3. In one embodiment, "high stringency" conditions refer to conditions of about 65°C and not more than about 1M Na⁺. It is understood that salt concentration, temperature

35 and/or length of incubation may be varied in either the first or second hybridization steps such that one

obtains the hybridizing nucleic acid molecules according to the invention. Conditions for hybridization of nucleic acids and calculations of T_m for nucleic acid hybrids are described in Sambrook et al. Molecular
5 Cloning: A Laboratory Manual Cold Spring Harbor Laboratory Press, New York. (1989).

The nucleic acids of the invention may hybridize to part or all of the polypeptide coding regions of AGP-1 as shown in SEQ ID NO: 1 and SEQ ID NO:
10 3, and therefore may be truncations or extensions of the nucleic acids in SEQ ID NO: 1 and SEQ ID NO: 3. Truncated or extended nucleic acids are encompassed by the invention provided that they retain one or more of the biological properties of AGP-1, such as stimulating
15 myelopoiesis, bone resorption or an inflammatory response. In one embodiment, the nucleic acid will encode a polypeptide of at least about 10 amino acids. In another embodiment, the nucleic acid will encode a polypeptide of at least about 20 amino acids. In yet
20 another embodiment, the nucleic acid will encode polypeptides of at least about 50 amino acids. The hybridizing nucleic acids may also include noncoding sequences located 5' and/or 3' to the AGP-1 coding regions. Noncoding sequences include regulatory regions
25 involved in AGP-1 expression, such as promoters, enhancer regions, translational initiation sites, transcription termination sites and the like.

In preferred embodiments, the nucleic acids of the invention encode mouse AGP-1 or human AGP-1. Mouse
30 AGP-1 is shown in Figure 1 and SEQ. ID. NO: 2 and human AGP-1 is shown in Figure 2 and SEQ. ID. NO: 4. Nucleic acids may encode a full-length form of AGP-1 which is a membrane-bound or soluble forms of AGP-1 lacking part or all of the transmembrane region. The predicted
35 transmembrane region for human AGP-1 includes residues 16-36 as shown in SEQ. ID. NO: 4. Deletions of part or

all these residues would be expected to produce soluble forms of AGP-1.

The nucleic acids of the invention will be linked with DNA sequences so as to express biologically active AGP-1. Sequences required for expression are known to those skilled in the art and include promoters and enhancer sequences for initiation of RNA synthesis, transcription termination sites, ribosome binding sites for the initiation of protein synthesis, and leader sequences for secretion. Sequences directing expression and secretion of AGP-1 may be homologous, i.e., those sequences in the genome involved in AGP-1 expression and secretion, or may be heterologous. A variety of plasmid vectors are available for expressing AGP-1 in host cells. One example is plasmid pDSR α described in PCT Application No. 90/14363 which may be used for expression in mammalian hosts. AGP-1 coding regions may also be modified by substitution of preferred codons for optimal expression in a given host. Codon usage in bacterial, plant, insect and mammalian host systems is known and may be exploited by one skilled in the art to optimize mRNA translation. In addition, vectors are available for the tissue-specific expression of AGP-1 in transgenic animals. Retroviral and adenovirus-based gene transfer vectors may also be used for the expression of AGP-1 in human cells for in vivo therapy (see PCT Application No. 86/00922).

Procaryotic and eucaryotic host cells expressing AGP-1 are also provided by the invention. Host cells include bacterial, yeast, plant, insect or mammalian cells. AGP-1 may also be produced in transgenic animals such as mice or goats. Plasmids and vectors containing the nucleic acids of the invention are introduced into appropriate host cells using transfection or transformation techniques known to one skilled in the art. Host cells may contain DNA

sequences encoding the full-length AGP-1 gene as shown in Figure 1. Host cells will also process AGP-1 encoded by the full-length gene to the mature form or produce the mature form without processing by expression of DNA sequences encoding same. Examples of mammalian host cells for AGP-1 expression include, but are not limited to COS, CHO α -, 293 and 3T3 cells.

The invention also provides AGP-1 as the product of procaryotic or eucaryotic expression of an exogenous DNA sequence, i.e., AGP-1 is recombinant AGP-1. Exogenous DNA sequences include cDNA, genomic DNA and synthetic DNA sequences. AGP-1 may be the product of bacterial, yeast, plant, insect or mammalian cells expression. AGP-1 produced in bacterial cells will have an N-terminal methionine residue. The invention also provides for a process of producing AGP-1 comprising growing procaryotic or eucaryotic host cells transformed or transfected with nucleic acids encoding AGP-1 and isolating polypeptide expression products of the nucleic acids.

Polypeptides which are mammalian AGP-1 or are derivatives thereof are encompassed by the invention. A derivative of AGP-1 refers to a polypeptide having an addition, deletion, insertion or substitution of one or more amino acids such that the resulting polypeptide has at least one of the biological activities of AGP-1. The derivative may be naturally occurring, such as a polypeptide product of an allelic variant or a mRNA splice variant, or it may be constructed using techniques available to one skilled in the art for manipulating and synthesizing nucleic acids.

AGP-1 polypeptides may be full-length polypeptides or fragments thereof which, in preferred embodiments, are at least about ten amino acids, at

least about 20 amino acids, or at least about 50 amino acids in length. AGP-1 full-length polypeptides and fragments preferably have the amino acid sequence in Figure 1 or 2 or a portion thereof. The polypeptides
5 may or may not have an amino terminal methionine residue.

Also included in the invention are AGP-1 polypeptides which have undergone post-translational modifications (e.g., addition of N-linked or O-linked
10 carbohydrate chains, processing of N-terminal or C-terminal ends), attachment of chemical moieties to the amino acid backbone, chemical modifications of N-linked or O-linked carbohydrate chains, and addition of an N-terminal methionine residue as a result of procaryotic
15 host cell expression. As mouse and human AGP-1 are encoded as transmembrane proteins, soluble forms of AGP-1 are also envisioned. Such soluble forms may be readily constructed by removal of the transmembrane region of the polypeptide. The polypeptides may also be
20 modified with a detectable label, such as an enzymatic, fluorescent, isotopic or affinity label to allow for detection and isolation of the protein.

AGP-1 chimeric proteins comprising part or all of an AGP-1 amino acid sequence fused to a heterologous
25 amino acid sequence are also included. The heterologous sequence may be any sequence which allows the resulting fusion protein to retain the activity of AGP-1. The heterologous sequences include, for example, immunoglobulin fusions, such as an Fc region of IgG,
30 which provide dimerization, or fusions to enzymes which provide a label for the polypeptide.

The polypeptides of the invention are isolated and purified from tissues and cell lines which express AGP-1 and from transformed host cells expressing AGP-1,
35 or purified from cell cultures containing the secreted protein. Isolated AGP-1 polypeptide is free from

association with human proteins and other cell constituents.

Also provided by the invention are chemically modified derivatives of AGP-1 which provide additional advantages such as increased stability, longer circulating time, or decreased immunogenicity (see for example U.S. Patent No. 4,179,337). The chemical moieties for derivitization may be selected from water soluble polymers such as polyethylene glycol, ethylene glycol/propylene glycol copolymers, carboxymethylcellulose, dextran, polyvinyl alcohol and the like. The polypeptides may be modified at random positions within the molecule, or at predetermined positions within the molecule and may include one, two, three or more attached chemical moieties.

A method for the purification of AGP-1 from natural sources (e.g. tissues and cell lines which normally express AGP-1) and from transfected host cells is also encompassed by the invention. The purification process may employ one or more standard protein purification steps in an appropriate order to obtain purified protein. The chromatography steps can include ion exchange, gel filtration, hydrophobic interaction, reverse phase, chromatofocusing, affinity chromatography employing an anti-AGP-1 antibody or biotin-streptavidin affinity complex and the like.

The invention also encompasses AGP-1 antagonists and the methods for obtaining them. An antagonist will reduce or eliminate one or more of the biological activities of AGP-1. As examples, an AGP-1 antagonist may act as an anti-inflammatory agent, or may act to inhibit bone resorption. AGP-1 antagonists include substances which bind to AGP-1 or to AGP-1 receptors in a manner to prevent normal ligand-receptor interaction and substances which regulate the expression

of AGP-1. Substances which bind to AGP-1 or to AGP-1 receptors include proteins, peptides, carbohydrates and small molecular weight organic compounds. Examples of protein inhibitors include anti-AGP-1 antibodies, anti-AGP-1 receptor antibodies and soluble forms of AGP-1 receptor comprising part or all of the extracellular domain of the AGP-1 receptor. Substances which regulate AGP-1 expression typically include nucleic acids which are complementary to nucleic acids encoding AGP-1 or AGP-1 receptors and which act as anti-sense regulators of expression.

Methods for indentifying compounds which interact with AGP-1 are also encompassed by the invention. The method comprises incubating AGP-1 with a compound under conditions which permit binding of the compound to AGP-1 and measuring the extent of binding. The compound may be substantially purified or present in a crude mixture. Binding compounds may be proteins, peptides, carbohydrates or small molecular weight organic compounds. The compounds may be further characterized by their ability to enhance or reduce AGP-1 biological activity and therefore act as AGP-1 agonists or as AGP-1 antagonists. Preferably, the method is used to identify AGP-1 antagonists.

Antibodies specifically binding the AGP-1 polypeptides of the invention are also encompassed by the invention. The antibodies may be produced by immunization with full-length membrane-bound AGP-1, soluble AGP-1, or a peptide fragment thereof, and the antibodies may be polyclonal or monoclonal. In addition, the antibodies of the invention may be recombinant, such as chimeric antibodies wherein the murine constant regions on light and heavy chains are replaced by human sequences, or CDR-grafted antibodies wherein only the complementary determining regions are

of murine origin. Antibodies of the invention may also be human antibodies prepared, for example, by immunization of transgenic animals capable of producing human antibodies (see, for example, PCT Application
5 No. WO93/12227). The antibodies are useful for detecting AGP-1 in biological samples, thereby allowing the identification of cells or tissues which produce AGP-1. In addition, antibodies which bind to AGP-1 and prevent receptor interaction may also be useful for
10 blocking the effects of AGP-1.

The invention also provides for pharmaceutical compositions comprising a therapeutically effective amount of the AGP-1 polypeptide of the invention
15 together with a pharmaceutically acceptable diluent, carrier, solubilizer, emulsifier, preservative and/or adjuvant. The invention also provides for pharmaceutical compositions comprising a therapeutically effective amount of an AGP-1 antagonist. The term
20 "therapeutically effective amount" means an amount which provides a therapeutic effect for a specified condition and route of administration. The composition may be in a liquid or lyophilized form and comprises a diluent (Tris, acetate or phosphate buffers) having various pH
25 values and ionic strengths, solubilizer such as Tween or Polysorbate, carriers such as human serum albumin or gelatin, preservatives such as thimerosal or benzyl alcohol, and antioxidants such as ascorbic acid or sodium metabisulfite. Also encompassed are compositions
30 comprising AGP-1 modified with water soluble polymers to increase solubility, stability, plasma half-life and bioavailability. Compositions may also comprise incorporation of AGP-1 into liposomes, microemulsions, micelles or vesicles for controlled delivery over an
35 extended period of time. Selection of a particular composition will depend upon a number of factors,

including the condition being treated, the route of administration and the pharmacokinetic parameters desired. A more extensive survey of component suitable for pharmaceutical compositions is found in Remington's
5 Pharmaceutical Sciences, 18th ed. A.R. Gennaro, ed. Mack, Easton, PA (1980).

Compositions of the invention may be administered by injection, either subcutaneous, intravenous or intramuscular, or by oral, nasal,
10 pulmonary or rectal administration. The route of administration eventually chosen will depend upon a number of factors and may be ascertained by one skilled in the art.

The invention also provides for pharmaceutical
15 compositions comprising a therapeutically effective amount of the nucleic acids of the invention together with a pharmaceutically acceptable adjuvant. Nucleic acid compositions will be suitable for the delivery of part or all of the APG-1 coding region and/or flanking
20 regions to cells and tissues as part of an anti-sense therapy regimen.

Hepatic expression of AGP-1 in transgenic mice resulted in marked myelopoiesis accompanied by an
25 increase in neutrophils and lymphocytes. Therefore, AGP-1 may be used to treat hematopoietic disorders that are associated with a decrease in the population of cells in bone marrow. In particular, AGP-1 may be used to treat conditions resulting in low white blood cell
30 levels, particularly reduced levels of neutrophils and lymphocytes. Such conditions may result from disease, injury or exposure to certain environmental agents known to suppress bone marrow levels. It is understood that AGP-1 may be administered alone or in combination with
35 other factors to treat hematopoietic disorders. In one embodiment, AGP-1 is used in conjunction with a

therapeutically effective amount of a factor which stimulates hematopoiesis. Such factors include erythropoietin (EPO), granulocyte colony stimulating factor (G-CSF), megakaryocyte growth and differentiation
5 factor (MGDF), granulocyte-macrophage stimulating factor (GM-CSF), stem cell factor (SCF), interleukin-3 (IL-3) and interleukin-6 (IL-6).

Hepatic expression of AGP-1 in transgenic mice resulted in increased inflammation and necrosis,
10 especially in the liver. This effect may be the result of a high local concentration of AGP-1 occurring in the liver during transgene expression. Thus, antagonists of AGP-1 may be used as anti-inflammatory agents which are administered to patients susceptible to or suffering
15 from an inflammatory condition. Inflammatory conditions include rheumatoid arthritis, systemic lupus erythematosus, psoriasis, systemic and localized amyloidosis, Sjogerns syndrome, sclerodoma, dermatomyositis, glomerulonephritis, and inflammation
20 arising from infections and parasitic diseases. AGP-1 antagonists which reduce or eliminate inflammation may be administered alone or in combination with a therapeutically effective amount of an anti-inflammtory agent such as a corticosteroid, a non-steroidal
25 anti-inflammatory agent (NSAID), or cyclosporin A. AGP-1 antagonists may also reduce or eliminate necrosis associated with an inflammatory condition.

AGP-1 is also involved in stimulation of osteoclasts which promote bone resorption through
30 mineralization of the bone matrix. Increase in bone resorption rates that exceed rates of bone formation can lead to various bone disorders including osteoporosis, osteomyelitis, hypercalcemia, osteopenia brought on by surgery or steroid administration, Paget's disease,
35 osteonecrosis, bone loss due to rheumatoid arthritis, periodontal bone loss, and osteolytic metastasis.

Antagonists of AGP-1 may be administered to patients suffering from disorders brought on by increased osteoclast activity and increased bone resorption. AGP-1 antagonists may be administered alone or in
5 combination with a therapeutically effective amount a bone growth promoting agent including bone morphogenic factors designated BMP-1 to BMP-12, transforming growth factor- β and TGF- β family members, interleukin-1 inhibitors, TNF α inhibitors, parathyroid hormone,
10 E series prostaglandins, bisphosphonates and bone-enhancing minerals such as fluoride and calcium.

The following examples are offered to more fully illustrate the invention, but are not construed as limiting the scope thereof.

15

EXAMPLE 1

Identification and Isolation of Murine and Human AGP-1
20 Genes

A. Murine AGP-1

Materials and method for cDNA cloning and analysis are described in Sambrook et.al. Molecular Cloning: A Laboratory Manual, 2d ed., Cold Spring Harbor
25 Laboratory Press (1989). A cDNA library was constructed using mRNA isolated from 5, 6, and 7 day post-5FU treated bone marrow from C57/B6 female mice. Mice were treated with 150mg/kg 5-fluorouracil (5FU),
30 intraperitoneally, on each of three consecutive days. On day 5, 6, and 7 post-5FU treatment both femurs and tibias were harvested, and plugs flushed with PBS. Bones were crushed with mortar and pestle and combined with the bone marrow plugs. The poly A+ mRNA was
35 purified using Fast Track mRNA Kit (Invitrogen, San Diego, CA) using the manufacturer's recommended

procedures. A random primed cDNA library was prepared using the Superscript Plasmid System (Gibco BRL, Gaithersburg, MD). A random cDNA primer containing an internal Not I restriction site was used to initiate
5 first strand synthesis and had the following double strand sequence:

5'-CCTCTGCGGCCGCTACANNNNNNNT-3' (SEQ ID NO: 5)
3'-pGGAGACGCCGCGA-5' (SEQ ID NO: 6)

10

The first strand cDNA synthesis reaction was assembled using 1µg of the mRNA and 150 ng of the Not I random primer. After second strand synthesis, the reaction products were extracted with the
15 phenol:chloroform:isoamyl alcohol mixture and ethanol precipitated. The double strand (ds) cDNA products were ligated to the following ds oligonucleotide adapter (Gibco BRL):

20 5'-TCGACCCACGCGTCCG-3' (SEQ ID NO: 7)
3'-GGGTGCGCAGGCp-5' (SEQ ID NO: 8)

After ligation the cDNA was digested to completion with Not 1, extracted with
25 phenol:chloroform:isoamyl alcohol (25:24:1 ratio) and ethanol precipitated. The resuspended cDNA was then size fractionated by gel filtration using the premade columns provided with the Superscript Plasmid System (Gibco BRL) as recommended by the manufacturer. The
30 fractions containing the largest cDNA products were ethanol precipitated and then directionally ligated into Not 1 and Sal 1 digested pMOB vector DNA (Strathmann et. al. Science 252, 802-808 (1991)). The ligated cDNA was introduced into electrocompetent XL1-Blue E. coli
35 (Stratagene, LaJolla, CA) by electroporation. Approximately 20,000 colonies were picked and arrayed into 96 well microtiter plates containing 200 µl of

L-broth, 7.5% glycerol, 50 µg/ml ampicillin and 12.5µg/ml tetracycline. The cultures were grown overnight at 37°C, a duplicate set of microtiter plates were made using a sterile 96 pin replicating tool, then
5 both sets were stored at -80°C for further analysis.

To sequence random murine 5FU-treated bone marrow cDNA clones, sequencing template was prepared by PCR amplification of cloned cDNA inserts using vector primers. Glycerol stocks of cDNA clones were thawed,
10 and small aliquots were diluted 1:25 in distilled water. Approximately 3.0 µl of diluted bacterial cultures were added to PCR reaction mixture (Boehringer-Mannheim) containing the following oligonucleotides:

15 5' TGTAACGACGGCCAGT 3' (SEQ ID NO: 9)
 5' CAGGAAACAGCTATGACC 3' (SEQ ID NO: 10).

The reactions were incubated in a thermocycler (Perkin-Elmer 9600) with the following cycle conditions:
20 94°C for 2 minutes; 94°C for 5 seconds, 50°C for 5 seconds and 72°C for 3 minutes for 30 cycles and then a final extension at 72°C for 4 minutes. After incubation in the thermocycler, the reactions were diluted with 2.0 ml of water. The amplified DNA
25 fragments were further purified using Centricon columns (Princeton Separations) using the manufacturer's recommended procedures. In some instances, low primer and deoxynucleoside triphosphate concentrations were used in the amplification reactions, and in those
30 instances, Centricon purification was not necessary. The PCR reaction products were sequenced on an Applied Biosystems 373A automated DNA sequencer using T3 primer:

5'-CAATTAACCCTCACTAAAGG-3' (SEQ ID NO: 11)

Taq dye-terminator reactions (Applied Biosystems) following the manufacturer's recommended procedures.

The resulting 5' nucleotide sequence obtained from randomly picked cDNA clones were translated and
5 then compared to the existing database of known protein sequences using a modified version of the FASTA program (Pearson, et. al. Meth. Enzymol. 183, 63-98 (1990)). Translated sequences were also analyzed for the presence of specific tumor necrosis factor superfamily motifs,
10 using the sequence profile method of Gribskov, et. al. (Proc. Natl. Acad. Sci. USA 83, 4355-4359 (1987)) as modified by Luethy et al. (Protein Science 3, 139-146 (1994)).

Using the FASTA and Profile search data, an
15 EST designated muAGP-EST1 was identified as a possible new member of the TNF family. The muAGP-EST1 clone contained an 864 bp insert with an open reading frame of about 90 amino acids which was found to have significant homology to pig lymphotoxin- α precursor (TNF- β) and
20 rabbit tumor necrosis factor precursor (TNF- α) (cachectin). The region compared showed an overlap of 63 amino acids and a 27% homology to TNF- β and a 71 amino acid overlap and 30% homology to TNF- α . Profile analysis using the TNF family profile yielded a
25 z score of 13.5, indicating that the muAGP-EST1 clone was encoding a possible new member of the TNF family.

To obtain a full-length clone, an internal EST database was searched for overlapping clones and two other murine EST clones were identified. One EST clone
30 designated muAGP-EST2 from a murine irradiated small intestine library gave a sequence which overlapped the sequence obtained from the muAGP-EST1 clone. The muAGP-EST2 clone was subsequently sequenced in its entirety. The insert was 3048 bp and contained an open
35 reading frame of 291 amino acids which was deduced to be the full-length AGP-1 sequence. The nucleotide sequence

and deduced amino acid of murine AGP-1 is shown in Figure 1.

B. Human AGP-1

5 A cDNA library was constructed using RNA from human bladder carcinoma cell line 5637 which had been stimulated with 20nM of PMA for about nine hours. For this library, mRNA was isolated from a membrane bound polysomal fraction of RNA (Mechler Methods in
10 Enzymology 152, 241-248 (1987)). The poly A+ mRNA fraction was isolated from the total RNA preparation by using the Fast Track mRNA Isolation Kit (InVitrogen) according to the manufacturer's recommended procedure. A directional random primed cDNA library was prepared
15 essentially as described for the 5-FU mouse bone marrow library above. The cDNA inserts were sequenced as described above for the mouse cDNA clones.

 The resulting 5' nucleotide sequences obtained from randomly picked cDNA clones were translated and
20 compared to the existing database of known protein sequences using a modified version of the FASTA program (Pearson et al. ibid). Translated sequences were also analysed for the presence of specific motifs found in the tumor necrosis factor superfamily using the sequence
25 profile method of Gribskov et.al. ibid as modified by Luethy et.al. ibid.

 Using the FASTA and Profile search data, an EST from the 5637 cell line cDNA library designated huAGP-EST1 was identified as a possible new member of
30 the TNF family. huAGP-EST1 contained an 446 bp insert with an open reading frame of about 84 amino acids. Translation of the huAGP-EST1 nucleotide sequence gave an amino acid sequence which was 77% identical to the deduced amino acid sequence of murine AGP-1 when
35 compared using FASTA analysis. This high degree of

sequence similarity identifies huAGP-EST1 as the human homolog of murine AGP-1.

To obtain a full-length clone, an internal EST database was searched for overlapping clones and one other murine EST clone was identified. This clone, designated huAGP-EST2, was from a human peripheral blood megakaryocyte cDNA library and had an insert of 1028 bp which overlapped the huAGP-EST1 clone. The overlapping clones had an open reading frame of 281 amino acids.

The full-length human AGP-1 was obtained as a composite of the sequences from the huAGP-EST1 and huAGP-EST2 clones. The nucleotide sequence and deduced amino acid sequence of human AGP-1 is shown in Figure 2.

15

EXAMPLE 2

Expression of AGP-1 in transgenic mice

20 A. PCR and subcloning

The TNF α -related clone muAGP-EST2 was used as template to PCR amplify the coding region for subcloning into an APOE-liver specific expression vector (Simonet et al. J. Clin. Invest. 94, 1310-1319 (1994), and PCT Application No. WO94/11675). The oligonucleotides used for amplification were:

5'-GAC TAG TCA GAC CTG GAC AGC AGT ATG CCT TC-3'
(SEQ ID NO: 12); and
30 5'-ATA AGA ATG CGG CCG CTA AAC TAT GGG TAC TTT AGG
GCT GTG TTT G-3' (SEQ ID NO: 13)

The conditions for PCR were: 94°C for 1 minute, followed by 25 cycles of 94°C for 20 sec, 63°C for 30 sec, and 74°C for 1 minute. The PCR reactions contained 1 x PFU buffer, 50 uM dNTPs, 20 pmol of each

oligo, 10 ng of DNA template and 2.5 units of PFU enzyme in a total volume of 50 ul. Following amplification, the samples were purified over Qiagen PCR columns and digested overnight with SpeI and NotI restriction
5 enzymes. The digested products were extracted and precipitated and subcloned into the ApoE promoter expression vector.

Ligations were transformed into *E. coli* strain DH5 α and colonies were miniprep for analysis of the
10 insert. Two clones containing the desired size insert were grown in 100ml TB cultures and plasmid DNA was prepared. The two clones were sent to sequencing to verify the authenticity of the insert. One was selected for microinjection to generate transgenic mice. This
15 transgene was designated HE-AGP.

B. Preparation of transgenic mice

For microinjection, the HE-AGP plasmid was purified through two rounds of CsCl. The plasmid was
20 digested with XhoI and Ase I, and the 3.4 kb transgene insert was purified on a 0.8% BRL ultrapure DNA agarose gel by electrophoresis onto NA 45 paper. The purified fragment was diluted to 1 ug/ml in 5 mM Tris, pH 7.4, 0.2 mM EDTA. Single-cell embryos from BDF1 x BDF1-bred
25 mice were injected essentially as described (Brinster et al., 1985), except that injection needles were beveled and siliconized before use. Embryos were cultured overnight in a CO₂ incubator and 15 to 20 two-cell embryos were transferred to the oviducts of
30 pseudopregnant CD1 female mice.

C. Screening of transgenic founders

Following term pregnancy, 105 offspring were obtained from implantation of microinjected embryos. Of
35 the 105 offspring, 17 were identified as transgenic founders by screening for the HE-AGP transgene in DNA

prepared from ear and tail biopsies. The PCR screening involved amplification of a 369 bp region of the human Apo E intron which was included in the expression vector. The oligos used for PCR amplification were:

5

5'-GCC TCT AGA AAG AGC TGG GAC-3' (SEQ. ID. NO: 14)

5'-CGC CGT GTT CCA TTT ATG AGC-3' (SEQ. ID. NO: 15)

The conditions for PCR were: 94°C for
10 2 minute, followed by 30 cycles of 94°C for 1 min, 63°C for 20 sec, and 72°C for 30 sec. The PCR reactions contained 1 x Taq buffer, 100 uM each dNTPs, 20 pmol of each oligo, 1 ul of DNA template extract and 0.5 units of taq enzyme in a total volume of 50 ul.

15

D. Preparation and analysis of total RNA for Northern analysis

At 8-10 weeks of age, 8 of the 17 transgenics (#'s 10, 27, 52, 53, 69, 72, 76 and 77) and 4 control
20 littermates (#'s 55, 56, 57, and 58) were sacrificed for necropsy and pathological analysis (See Example 3). Liver was isolated from the remaining 9 founders (#'s 25, 42, 44, 45, 48, 50, 67, 74, and 75) by partial
25 hepatectomy. For partial hepatectomy, the mice were anesthetized with avertin and a lobe of liver was surgically removed. Total cellular RNA was isolated from livers of all transgenic founders, and 5 negative control littermates as described (McDonald et al. (1987)). Northern blot analysis was performed on these
30 samples to assess the level of transgene expression. Approximately 10ug of total RNA from each animal liver was resolved by electrophoresis denaturing gels (Ogden et al. (1987)), then transferred to HYBOND-N nylon membrane (Amersham), and probed with ³²P dCTP-labelled
35 pB1.1 insert DNA. Hybridization was performed overnight at 42°C in 50% Formamide, 5 x SSPE, 0.5% SDS, 5 x

Denhardt's solution, 100 ug/ml denatured salmon sperm DNA and $2-4 \times 10^6$ cpm of labeled probe/ml of hybridization buffer. Following hybridization, blots were washed twice in $2 \times \text{SSC}$, 0.1% SDS at room temperature for 5 min each, and then twice in $0.1 \times \text{SSC}$, 0.1% SDS at 55°C for 5-10 min each. Expression of the transgene in founder and control littermates was determined following autoradiography.

The Northern blot data indicate that 13 of the transgenic founders express detectable levels of the transgene mRNA (animal #'s 10, 42, 44, 45, 48, 50, 52, 53, 67, 69, 74, 75 and 76). The negative control mice expressed no transgene-related mRNA. The highest expressing founders from the group that were necropsied were #'s 52, 69 and 76. The highest expressing animals from the remaining group of founder's were #'s 42, 45, 67, and 75. Six of the founder's that were analyzed by hepatectomy were subsequently bred to generate F1 offspring for further analysis.

EXAMPLE 3

Pathology Analysis of Transgenic Mice Expressing AGP-1

A. Necropsy

Mice from two separate studies were examined. In the first study, five BDF1 female mice which were founder transgenics for the murine AGP-1 molecule targeted to the liver via an apolipoprotein E promoter as well as four male non-transgenic littermate mice were necropsied for phenotypic analysis. In the second study, twelve BDF1 mice (nine females and three males) which were F1 transgenics for the murine AGP molecule targeted to the liver via an apolipoprotein E promoter as well as four female non-transgenic littermate mice

were necropsied for phenotypic analysis. In both studies, all mice were injected with BrdU one hour prior to harvest and sacrificed. Body and liver, spleen, kidney, stomach, and thymus weights were taken, blood
5 was drawn for hematology and serum chemistries, and liver, spleen, lung, brain, heart, kidney, adrenal, stomach, small intestine, pancreas, cecum, colon, mesenteric lymph node, skin, mammary gland, trachea, esophagus, thyroid, parathyroid, salivary gland, urinary
10 bladder, ovary or testis, uterus or seminal vesicle, bone, and bone marrow were examined were harvested for histologic analysis and BrdU labeling.

B. Histology and Histochemistry

15 Sections of liver, spleen, lung, brain, heart, kidney, adrenal, stomach, small intestine, pancreas, cecum, colon, mesenteric lymph node, skin, mammary gland, trachea, esophagus, thyroid, parathyroid, salivary gland, urinary bladder, ovary or testis, uterus
20 or seminal vesicle, bone, and bone marrow from AGP-1 transgenic and non-transgenic mice were fixed overnight in 10% neutral buffered zinc formalin (Anatech, Battle Creek, Michigan), paraffin embedded, sectioned at 3 μ m, and stained with hematoxylin and eosin (H&E) for routine
25 histologic examination. In addition, sections of bone were stained for tartrate resistant acid phosphatase (TRAP) to highlight osteoclasts around bony trabeculae in marrow spaces.

C. Immunohistochemistry

30 Immunohistochemical staining was done on 4 μ m thick paraffin embedded sections using an automated TechMate Immunostainer (BioTek Solutions, Santa Barbara, CA). For BrdU immunostaining, sections were first
35 digested with 0.1% protease (Sigma Chemical, St. Louis, MO) followed by 2N HCl. BrdU was detected with a rat

monoclonal antibody (MAb) to BrdU (Accurate Chemical, Westbury, NY) followed by a biotinylated anti-rabbit/anti-mouse secondary cocktail (BioTek) and an ABC tertiary coupled to alkaline phosphatase (BioTek). The staining reaction was visualized with BioTek Red chromagen (BioTek). For myeloperoxidase immunostaining, sections were stained with rabbit polyclonal antisera directed at human myeloperoxidase (Dako, Carpinteria, CA), followed by a biotinylated anti-rabbit/anti-mouse secondary cocktail (BioTek) and avidin-biotin complex (ABC) tertiary coupled to horseradish peroxidase. The staining reaction was visualized with diaminobenzidine (DAB, Sigma).

15 D. Gross Pathology Findings

The livers from two transgenic founder mice (#s 69 and 76) and two F1 transgenic mice (#s 75-13 and 75-18) were significantly increased in size and weight (8.42 ± 1.26 SD % of body weight vs. 5.33 ± 0.89 SD % of body weight in non-transgenic control mice) and were pale green-tan and more friable than normal. These four mice also had a significant increase in splenic weight (1.14 ± 0.12 SD % of body weight vs. 0.41 ± 0.09 SD % body weight in non-transgenic control mice). These results are summarized in Table 1.

E. Clinical Pathology Findings

The four transgenic mice with enlarged livers (founder #s 69 and 76 and F1 #s 75-13 and 75-18) had marked and significant increases in total serum bilirubin and alkaline phosphatase levels, with moderate but significant increases in hepatic transaminase (alanine aminotransferase (ALT) and aspartate aminotransferase (AST)) levels. The four transgenic mice had a mean total bilirubin level of 4.33 ± 5.32 SD mg/dl while non-transgenic control mice had a

mean total bilirubin level of 0.16 ± 0.05 SD mg/dl. The mean serum alkaline phosphatase level in these four transgenic mice was 994.5 ± 353.1 SD IU/l vs. 165.3 ± 53.2 SD IU/l in non-transgenic control mice. The mean ALT level in these four transgenic mice was 247.3 ± 89.8 SD IU/l vs. 78.1 ± 43.2 SD IU/l in non-transgenic control mice while the mean AST level in these four transgenic mice was 350.5 ± 135.6 SD IU/l vs. 132.5 ± 84.9 SD IU/l in non-transgenic control mice. All of these results are summarized in table 1.

F. Histopathologic Findings

H&E and BrdU stained sections of liver, spleen, lung, brain, heart, kidney, adrenal, stomach, small intestine, pancreas, cecum, colon, mesenteric lymph node, skin, mammary gland, trachea, esophagus, thyroid, parathyroid, salivary gland, urinary bladder, ovary or testis, uterus or seminal vesicle, bone, and bone marrow were examined from the 17 HE-AGP-1 transgenic mice and 8 non-transgenic control littermates. Myeloperoxidase stained sections of spleen and bone marrow as well as tartrate resistant acid phosphatase (TRAP) stained sections of bone were also examined from all mice. Major histologic changes in the transgenic mice included marked periportal inflammation and bile duct hyperplasia with scattered multifocal to coalescing areas of hepatocellular necrosis in transgenic mice #s 69 and 76 (founders) and #s 75-13 and 75-18 (F1s) (Figure 3). All four of these transgenic mice also had enlarged spleens primarily due to increased red pulp myelopoiesis and to a lesser extent, lymphoid hyperplasia (Figure 4). These four transgenic mice also appeared to have increased numbers of TRAP positive osteoclasts lining bony trabeculae in peripheral diaphyseal marrow compared to non-transgenic control mice (Figure 5). Transgenic mice also exhibited

increased intravascular neutrophils, and small atrophic/hypoplastic uteri (only founder transgenics #s 69 and 76). The two founder transgenic mice (#s 69 and 76) also exhibited moderate peritoneal mixed inflammatory cellular infiltration.

G. Summary of Pathologic Findings in Transgenic Mice Overexpressing AGP-1

Four of the HE-AGP-1 transgenic mice (founder nos. 69 and 76 and F1 nos. 75-13 and 75-18) had relatively severe phenotypic alterations, particularly in their livers with marked cholangiohepatitis, bile duct hyperplasia and hepatic necrosis. Accompanying these hepatic histologic abnormalities in these four transgenic mice was evidence of liver dysfunction with marked elevations in total serum bilirubin and alkaline phosphatase with moderate elevations in serum transaminases. In addition to hepatic findings, these four transgenic mice also exhibited increased myelopoiesis, with a less prominent increase in circulating platelets. Founder mouse #69 had a circulating neutrophilia while all transgenic mice had a moderate increase in circulating lymphocytes. Evidence of peritoneal inflammation was also seen in the two founder transgenic mice with marked hepatic inflammation. Two of the other HEAGP founder transgenic mice, #'s 52 and 53, also had evidence of mild cholangiohepatitis, and a mild to moderate increase in myelopoiesis and neutrophilia, suggesting that these two mice were producing the transgenic AGP-1 protein at a lower level than founder mice #s 69 and 76 were. In addition to hepatic findings, at least four of the transgenic mice exhibited a marked increase in splenic myelopoiesis and moderate lymphoid hyperplasia as well as exhibiting an apparent increase in TRAP+ osteoclasts lining bony trabeculae in the bone marrow. All of these

findings suggest that the AGP protein plays a role in inflammation, myelopoiesis, and bone resorption (osteoclasia).

5 **Table 1 Selected Organ Weights and Serum Chemistries in HE-AGP-1 Transgenic Mice**

	HEAGP Transgenic Mice (n=4)	Non- transgenic Mice (n=8)	p value
Liver Weight as % of Body Weight	8.42 ± 1.26 SD	5.33 ± 0.89 SD	0.0006
Spleen Weight as % Body Weight	1.14 ± 0.12 SD	0.41 ± 0.09 SD	<0.0001
Total Bilirubin (mg/dl)	4.33 ± 5.32 SD	0.16 ± 0.05 SD	0.04
Alkaline Phosphatase (IU/l)	994.5 ± 353.1 SD	165.3 ± 53.2 SD	<0.0001
Alanine Aminotransferase (ALT) (IU/l)	247.3 ± 89.8 SD	78.1 ± 43.2 SD	0.001
Aspartate Aminotransferase (AST) (IU/l)	350.5 ± 135.6 SD	132.5 ± 84.9 SD	0.006

SEQUENCE LISTING

- 5 (1) GENERAL INFORMATION:
- (i) APPLICANT: Johnson, Merrie Jo
Simonet, William S.
Danilenko, Dmitry M.
- 10 (ii) TITLE OF INVENTION: TUMOR NECROSIS FACTOR-RELATED
POLYPEPTIDE
- (iii) NUMBER OF SEQUENCES: 15
- 15 (iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Amgen Inc.
(B) STREET: 1840 Dehavilland Drive
(C) CITY: Thousand Oaks
(D) STATE: California
20 (E) COUNTRY: U.S.A.
(F) ZIP: 91320
- (v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
25 (B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30
- (vi) CURRENT APPLICATION DATA:
30 (A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:
- (viii) ATTORNEY/AGENT INFORMATION:
3 (A) NAME: Winter, Robert B.
(C) REFERENCE/DOCKET NUMBER: A-410
- (2) INFORMATION FOR SEQ ID NO:1:
- 40 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3048 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
45 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- 50 (ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 245..1120
- 55 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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	GTTTCACTTT TGGTCTCAAC AGTAAAAAGA AACTGCATGG GCACTCCGCC TTCTAACTGT	180
5	GACCTTCTCA GGCAGTGTG CTGGGCTGCA AGTCTGCATT GGAAGTCAG ACCTGGACAG	240
	CAGT ATG CCT TCC TCA GGG GCC CTG AAG GAC CTC AGC TTC AGT CAG CAC	289
	Met Pro Ser Ser Gly Ala Leu Lys Asp Leu Ser Phe Ser Gln His	
	1 5 10 15	
10	TTC AGG ATG ATG GTG ATT TGC ATA GTG CTC CTG CAG GTG CTC CTG CAG	337
	Phe Arg Met Met Val Ile Cys Ile Val Leu Leu Gln Val Leu Leu Gln	
	20 25 30	
15	GCT GTG TCT GTG GCT GTG ACT TAC ATG TAC TTC ACC AAC GAG ATG AAG	385
	Ala Val Ser Val Ala Val Thr Tyr Met Tyr Phe Thr Asn Glu Met Lys	
	35 40 45	
20	CAG CTG CAG GAC AAT TAC TCC AAA ATT GGA CTA GCT TGC TTC TCA AAG	433
	Gln Leu Gln Asp Asn Tyr Ser Lys Ile Gly Leu Ala Cys Phe Ser Lys	
	50 55 60	
25	ACG GAT GAG GAT TTC TGG GAC TCC ACT GAT GGA GAG ATC TTG AAC AGA	481
	Thr Asp Glu Asp Phe Trp Asp Ser Thr Asp Gly Glu Ile Leu Asn Arg	
	65 70 75	
30	CCC TGC TTG CAG GTT AAG AGG CAA CTG TAT CAG CTC ATT GAA GAG GTG	529
	Pro Cys Leu Gln Val Lys Arg Gln Leu Tyr Gln Leu Ile Glu Glu Val	
	80 85 90 95	
35	ACT TTG AGA ACC TTT CAG GAC ACC ATT TCT ACA GTT CCA GAA AAG CAG	577
	Thr Leu Arg Thr Phe Gln Asp Thr Ile Ser Thr Val Pro Glu Lys Gln	
	100 105 110	
40	CTA AGT ACT CCT CCC TTG CCC AGA GGT GGA AGA CCT CAG AAA GTG GCA	625
	Leu Ser Thr Pro Pro Leu Pro Arg Gly Gly Arg Pro Gln Lys Val Ala	
	115 120 125	
45	GCT CAC ATT ACT GGG ATC ACT CGG AGA AGC AAC TCA GCT TTA ATT CCA	673
	Ala His Ile Thr Gly Ile Thr Arg Arg Ser Asn Ser Ala Leu Ile Pro	
	130 135 140	
50	ATC TCC AAG GAT GGA AAG ACC TTA GGC CAG AAG ATT GAA TCC TGG GAG	721
	Ile Ser Lys Asp Gly Lys Thr Leu Gly Gln Lys Ile Glu Ser Trp Glu	
	145 150 155	
55	TCC TCT CGG AAA GGG CAT TCA TTT CTC AAC CAC GTG CTC TTT AGG AAT	769
	Ser Ser Arg Lys Gly His Ser Phe Leu Asn His Val Leu Phe Arg Asn	
	160 165 170 175	
60	GGA GAG CTG GTC ATC GAG CAG GAG GGC CTG TAT TAC ATC TAT TCC CAA	817
	Gly Glu Leu Val Ile Glu Gln Glu Gly Leu Tyr Tyr Ile Tyr Ser Gln	
	180 185 190	
65	ACA TAC TTC CGA TTT CAG GAA GCT GAA GAC GCT TCC AAG ATG GTC TCA	865
	Thr Tyr Phe Arg Phe Gln Glu Ala Glu Asp Ala Ser Lys Met Val Ser	
	195 200 205	

	AAG GAC AAG GTG AGA ACC AAA CAG CTG GTG CAG TAC ATC TAC AAG TAC	913
	Lys Asp Lys Val Arg Thr Lys Gln Leu Val Gln Tyr Ile Tyr Lys Tyr	
	210 215 220	
5	ACC AGC TAT CCG GAT CCC ATA GTG CTC ATG AAG AGC GCC AGA AAC AGC	961
	Thr Ser Tyr Pro Asp Pro Ile Val Leu Met Lys Ser Ala Arg Asn Ser	
	225 230 235	
10	TGT TGG TCC AGA GAT GCC GAG TAC GGA CTG TAC TCC ATC TAT CAG GGA	1009
	Cys Trp Ser Arg Asp Ala Glu Tyr Gly Leu Tyr Ser Ile Tyr Gln Gly	
	240 245 250 255	
15	GGA TTG TTC GAG CTA AAA AAA AAT GAC AGG ATT TTT GTT TCT GTG ACA	1057
	Gly Leu Phe Glu Leu Lys Lys Asn Asp Arg Ile Phe Val Ser Val Thr	
	260 265 270	
	AAT GAA CAT TTG ATG GAC CTG GAT CAA GAA GCC AGC TTC TTT GGA GCC	1105
	Asn Glu His Leu Met Asp Leu Asp Gln Glu Ala Ser Phe Phe Gly Ala	
	275 280 285	
20	TTT TTA ATT AAC TAA ATGACCAGTA AAGATCAAAC ACAGCCCTAA AGTACCCAGT	1160
	Phe Leu Ile Asn *	
	290	
25	AATCTTCTAG GTTGAAGGCA TGCCTGGAAA GCGACTGAAC TGGTTAGGAT ATGGCCTGGC	1220
	TGTAGAAACC TCAGGACAGA TGTGACAGAA AGGCAGCTGG AACTCAGCAG CGACAGGCCA	1280
30	ACAGTCCAGC CACAGACACT TTCGGTGTTT CATCGAGAGA CTTGCTTTCT TTCCGCAAAA	1340
	TGAGATCACT GTAGCCTTTC AATGATCTAC CTGGTATCAG TTTGCAGAGA TCTAGAAGAC	1400
	GTCCAGTTTC TAAATATTTA TGCAACAATT GACAATTTTC ACCTTTGTTA TCTGGTCCAG	1460
35	GGGTGTAAAG CCAAGTGCTC ACAGGCTGTG TGCAGACCAG GATAGCTATG AATGCAGGTC	1520
	AGCATAAAAA TCACAGAATA TCTCACCTAC CAAATCAGAG TGGGTGTGCC CCTGTGTGTA	1580
40	TATGCGTGTC TGTGTGTGTG TGCATGTATG TGTGTGTGTG TGTGACTGTT CTTTATGGTA	1640
	ACTGGTTATG TTTTCTCAA GTGAAAAACA TAACTCTATA CATGATAACA TAATATCCCA	1700
	TCATCAGTGG AACCTTGCCC AAAGAATGTA TGAAATCTCC AGGCAATGAA TGAGGGCAGC	1760
45	CCAAGAAAGA GGCCCGCAGA GCCATACCAC AGGGCTGCCC CACCCTGCTG GAGCTCAGAT	1820
	CCTGCCACTG CTGCAGGCCC TGGGTACCAG GTGTAGAGTT GGAGGAGGTC TTGCCTGTGG	1880
50	GTCTAGGTCT TTGGTGCCTA CCTCCTTGAT ATGGCCCCAG TCCTCCTTTG CTTGTTTGCT	1940
	AGTTTTATCA TGTTTCCCAG GCCGGCCTCA AGTCCAATAT GTAGTCAAGA GTGATCTCTA	2000
	ACTGTGCAAC CTCCTGCCTC CAAGATCTGC TGAGATTATA GGCATGTGCC CCCCTGTCTG	2060
55	ATTTGTGTAG AGCCAGGCTT CTTGTACATG TGACAACCAT GCCACCCTCA GCTCTGTCCC	2120
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	TGCAACAGTG AAGAATTTGC TCTGACTTTC AGGATAAAGT TTGAACTAGG TTCACCATGC	2240
	TTGCTTTGTC CAGATTGCGA CTGTCACCCA GTCCTCTGGC TCTTCCATCT GTCTGTCCAC	2300
5	TCCACCTACC AAGATGTTGA ACACTTGTTT TTTTAAAGAT GTTGGTGCCT GGAGTTTCAT	2360
	TAGAGTAACA CAAAATAAC TAAAACCAA CAACTCCAAA GGAGCCCATA TGTGTTTTAA	2420
10	TGAAACATTT TTTAAGCCTA TTGGGGGCCT GAAGAGATTG CTCAGAGGAA AACAGCACTT	2480
	CCAGAGGACC CAGGTTCAT TCTCATCGCT GATGTGATAG TTAACAGCTG TAACTTCAGT	2540
	TCCAAGGGGT CTGACTTTCT GCCCTTTGCT TGCAATGCAT GTATGTGATA CACAGACATA	2600
15	CATTCTGACA AAATATATCC ATACACAAAA GTATTTTTTT AAAAGCTTAT TTGAATGTAA	2660
	GAGTATGGCT AGCTGTCACT TCTGATACCC CTTCTTATTT TTTTATGACT CAAGCCCTTA	2720
20	TAAACTAGCA AATAGAAGTC ACAGCTACCA CTTGAATATA AGCACTTGAA TACCTCCTCT	2780
	CACTAGAATA CAACATAGCT TAATAGTAAA AATCTTGCCT TAGTAAAGTA CTTGCATGTC	2840
	ATGTCTACAT GAACCAAATG AATGTATTAA TTAATAATAG ACATAATGAT CACATCGGAA	2900
25	AGGCTGTGAG AAATAATGGA GAACATTTGA AAGCTCAAGA TGAAGGGGAA AGGCACTTGT	2960
	CAAAAATCTT GACAACCTGA ATTTGACCTT TGGCAGGGCT GAAAATAAA CCCAGGGTCT	3020
30	TACTCCCAGT AGGCATGAAC TCCCCCCT	3048

(2) INFORMATION FOR SEQ ID NO:2:

35	(i) SEQUENCE CHARACTERISTICS:
	(A) LENGTH: 292 amino acids
	(B) TYPE: amino acid
	(D) TOPOLOGY: linear
40	(ii) MOLECULE TYPE: protein
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:
45	Met Pro Ser Ser Gly Ala Leu Lys Asp Leu Ser Phe Ser Gln His Phe 1 5 10 15
	Arg Met Met Val Ile Cys Ile Val Leu Leu Gln Val Leu Leu Gln Ala 20 25 30
50	Val Ser Val Ala Val Thr Tyr Met Tyr Phe Thr Asn Glu Met Lys Gln 35 40 45
	Leu Gln Asp Asn Tyr Ser Lys Ile Gly Leu Ala Cys Phe Ser Lys Thr 50 55 60
55	Asp Glu Asp Phe Trp Asp Ser Thr Asp Gly Glu Ile Leu Asn Arg Pro 65 70 75 80

	Cys	Leu	Gln	Val	Lys	Arg	Gln	Leu	Tyr	Gln	Leu	Ile	Glu	Glu	Val	Thr	
					85					90					95		
5	Leu	Arg	Thr	Phe	Gln	Asp	Thr	Ile	Ser	Thr	Val	Pro	Glu	Lys	Gln	Leu	
				100					105					110			
	Ser	Thr	Pro	Pro	Leu	Pro	Arg	Gly	Gly	Arg	Pro	Gln	Lys	Val	Ala	Ala	
				115				120					125				
10	His	Ile	Thr	Gly	Ile	Thr	Arg	Arg	Ser	Asn	Ser	Ala	Leu	Ile	Pro	Ile	
				130			135					140					
	Ser	Lys	Asp	Gly	Lys	Thr	Leu	Gly	Gln	Lys	Ile	Glu	Ser	Trp	Glu	Ser	
	145					150					155				160		
15	Ser	Arg	Lys	Gly	His	Ser	Phe	Leu	Asn	His	Val	Leu	Phe	Arg	Asn	Gly	
					165					170					175		
	Glu	Leu	Val	Ile	Glu	Gln	Glu	Gly	Leu	Tyr	Tyr	Ile	Tyr	Ser	Gln	Thr	
20				180					185					190			
	Tyr	Phe	Arg	Phe	Gln	Glu	Ala	Glu	Asp	Ala	Ser	Lys	Met	Val	Ser	Lys	
			195					200					205				
25	Asp	Lys	Val	Arg	Thr	Lys	Gln	Leu	Val	Gln	Tyr	Ile	Tyr	Lys	Tyr	Thr	
		210					215					220					
	Ser	Tyr	Pro	Asp	Pro	Ile	Val	Leu	Met	Lys	Ser	Ala	Arg	Asn	Ser	Cys	
	225					230					235				240		
30	Trp	Ser	Arg	Asp	Ala	Glu	Tyr	Gly	Leu	Tyr	Ser	Ile	Tyr	Gln	Gly	Gly	
					245					250					255		
	Leu	Phe	Glu	Leu	Lys	Lys	Asn	Asp	Arg	Ile	Phe	Val	Ser	Val	Thr	Asn	
35				260					265					270			
	Glu	His	Leu	Met	Asp	Leu	Asp	Gln	Glu	Ala	Ser	Phe	Phe	Gly	Ala	Phe	
			275					280					285				
40	Leu	Ile	Asn	*													
		290															

(2) INFORMATION FOR SEQ ID NO:3:

- 45 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 1060 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: double
 (D) TOPOLOGY: linear
- 50 (ii) MOLECULE TYPE: cDNA
- 55 (ix) FEATURE:
 (A) NAME/KEY: CDS
 (B) LOCATION: 35..880

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

	GGCTGACTTA CAGCAGTCAG ACTCTGACAG GATC ATG GCT ATG ATG GAG GTC	52
	Met Ala Met Met Glu Val	
5	1 5	
	CAG GGG GGA CCC AGC CTG GGA CAG ACC TGC GTG CTG ATC GTG ATC TTC	100
	Gln Gly Gly Pro Ser Leu Gly Gln Thr Cys Val Leu Ile Val Ile Phe	
	10 15 20	
10	ACA GTG CTC CTG CAG TCT CTC TGT GTG GCT GTA ACT TAC GTG TAC TTT	148
	Thr Val Leu Leu Gln Ser Leu Cys Val Ala Val Thr Tyr Val Tyr Phe	
	25 30 35	
15	ACC AAC GAG CTG AAG CAG ATG CAG GAC AAG TAC TCC AAA AGT GGC ATT	196
	Thr Asn Glu Leu Lys Gln Met Gln Asp Lys Tyr Ser Lys Ser Gly Ile	
	40 45 50	
20	GCT TGT TTC TTA AAA GAA GAT GAC AGT TAT TGG GAC CCC AAT GAC GAA	244
	Ala Cys Phe Leu Lys Glu Asp Asp Ser Tyr Trp Asp Pro Asn Asp Glu	
	55 60 65 70	
	GAG AGT ATG AAC AGC CCC TGC TGG CAA GTC AAG TGG CAA CTC CGT CAG	292
	Glu Ser Met Asn Ser Pro Cys Trp Gln Val Lys Trp Gln Leu Arg Gln	
25	75 80 85	
	CTC GTT AGA AAG ATG ATT TTG AGA ACC TCT GAG GAA ACC ATT TCT ACA	340
	Leu Val Arg Lys Met Ile Leu Arg Thr Ser Glu Glu Thr Ile Ser Thr	
	90 95 100	
30	GTT CAA GAA AAG CAA CAA AAT ATT TCT CCC CTA GTG AGA GAA AGA GGT	388
	Val Gln Glu Lys Gln Gln Asn Ile Ser Pro Leu Val Arg Glu Arg Gly	
	105 110 115	
35	CCT CAG AGA GTA GCA GCT CAC ATA ACT GGG ACC AGA GGA AGA AGC AAC	436
	Pro Gln Arg Val Ala Ala His Ile Thr Gly Thr Arg Gly Arg Ser Asn	
	120 125 130	
40	ACA TTG TCT TCT CCA AAC TCC AAG AAT GAA AAG GCT CTG GGC CGC AAA	484
	Thr Leu Ser Ser Pro Asn Ser Lys Asn Glu Lys Ala Leu Gly Arg Lys	
	135 140 145 150	
	ATA AAC TCC TGG GAA TCA TCA AGG AGT GGG CAT TCA TTC CTG AGC AAC	532
	Ile Asn Ser Trp Glu Ser Ser Arg Ser Gly His Ser Phe Leu Ser Asn	
45	155 160 165	
	TTG CAC TTG AGG AAT GGT GAA CTG GTC ATC CAT GAA AAA GGG TTT TAC	580
	Leu His Leu Arg Asn Gly Glu Leu Val Ile His Glu Lys Gly Phe Tyr	
	170 175 180	
50	TAC ATC TAT TCC CAA ACA TAC TTT CGA TTT CAG GAG GAA ATA AAA GAA	628
	Tyr Ile Tyr Ser Gln Thr Tyr Phe Arg Phe Gln Glu Glu Ile Lys Glu	
	185 190 195	
55	AAC ACA AAG AAC GAC AAA CAA ATG GTC CAA TAT ATT TAC AAA TAC ACA	676
	Asn Thr Lys Asn Asp Lys Gln Met Val Gln Tyr Ile Tyr Lys Tyr Thr	
	200 205 210	

	AGT TAT CCT GAC CCT ATA TTG TTG ATG AAA AGT GCT AGA AAT AGT TGT	724
	Ser Tyr Pro Asp Pro Ile Leu Leu M t Lys Ser Ala Arg Asn Ser Cys	
	215 220 225 230	
5	TGG TCT AAA GAT GCA GAA TAT GGA CTC TAT TCC ATC TAT CAA GGG GGA	772
	Trp Ser Lys Asp Ala Glu Tyr Gly Leu Tyr Ser Ile Tyr Gln Gly Gly	
	235 240 245	
10	ATA TTT GAG CTT AAG GAA AAT GAC AGA ATT TTT GTT TCT GTA ACA AAT	820
	Ile Phe Glu Leu Lys Glu Asn Asp Arg Ile Phe Val Ser Val Thr Asn	
	250 255 260	
15	GAG CAC TTG ATA GAC ATG GAC CAT GAA GCC AGT TTT TTC GGG GCC TTT	868
	Glu His Leu Ile Asp Met Asp His Glu Ala Ser Phe Phe Gly Ala Phe	
	265 270 275	
	TTA GTT GGC TAA CTGACCTGGA AAGAAAAAGC AATAACCTCA AAGTGACTAT	920
	Leu Val Gly *	
	280	
20	TCAGTTTTCA GGATGATACA CTATGAAGAT GTTTCAAAAA ATCTGACCAA AACAAACAAA	980
	CAGAAAACAG AAAACAAAAA AACCTCTATG CAATCTGAGT AGAGCAGCCA CAACCAAAAT	1040
25	TGTATACAAC ACACCATGTA	1060

(2) INFORMATION FOR SEQ ID NO:4:

30	(i) SEQUENCE CHARACTERISTICS:	
	(A) LENGTH: 282 amino acids	
	(B) TYPE: amino acid	
	(D) TOPOLOGY: linear	
35	(ii) MOLECULE TYPE: protein	
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:	
40	Met Ala Met Met Glu Val Gln Gly Gly Pro Ser Leu Gly Gln Thr Cys	
	1 5 10 15	
	Val Leu Ile Val Ile Phe Thr Val Leu Leu Gln Ser Leu Cys Val Ala	
	20 25 30	
45	Val Thr Tyr Val Tyr Phe Thr Asn Glu Leu Lys Gln Met Gln Asp Lys	
	35 40 45	
	Tyr Ser Lys Ser Gly Ile Ala Cys Phe Leu Lys Glu Asp Asp Ser Tyr	
	50 55 60	
50	Trp Asp Pro Asn Asp Glu Glu Ser Met Asn Ser Pro Cys Trp Gln Val	
	65 70 75 80	
55	Lys Trp Gln Leu Arg Gln Leu Val Arg Lys Met Ile Leu Arg Thr Ser	
	85 90 95	
	Glu Glu Thr Ile Ser Thr Val Gln Glu Lys Gln Gln Asn Ile Ser Pro	
	100 105 110	

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Leu Val Arg Glu Arg Gly Pro Gln Arg Val Ala Ala His Ile Thr Gly
 115 120 125
 5 Thr Arg Gly Arg Ser Asn Thr Leu Ser Ser Pro Asn Ser Lys Asn Glu
 130 135 140
 Lys Ala Leu Gly Arg Lys Ile Asn Ser Trp Glu Ser Ser Arg Ser Gly
 145 150 155 160
 10 His Ser Phe Leu Ser Asn Leu His Leu Arg Asn Gly Glu Leu Val Ile
 165 170 175
 His Glu Lys Gly Phe Tyr Tyr Ile Tyr Ser Gln Thr Tyr Phe Arg Phe
 180 185 190
 Gln Glu Glu Ile Lys Glu Asn Thr Lys Asn Asp Lys Gln Met Val Gln
 195 200 205
 20 Tyr Ile Tyr Lys Tyr Thr Ser Tyr Pro Asp Pro Ile Leu Leu Met Lys
 210 215 220
 Ser Ala Arg Asn Ser Cys Trp Ser Lys Asp Ala Glu Tyr Gly Leu Tyr
 225 230 235 240
 25 Ser Ile Tyr Gln Gly Gly Ile Phe Glu Leu Lys Glu Asn Asp Arg Ile
 245 250 255
 Phe Val Ser Val Thr Asn Glu His Leu Ile Asp Met Asp His Glu Ala
 260 265 270
 30 Ser Phe Phe Gly Ala Phe Leu Val Gly *
 275 280

35 (2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 26 base pairs
 (B) TYPE: nucleic acid
 40 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

45 (ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

50 CCTCTGCGGC CGCTACANNN NNNNT

26

(2) INFORMATION FOR SEQ ID NO:6:

55 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 14 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

5

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

10 AGCGGCCGCA GAGG

14

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

15 (A) LENGTH: 16 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

20 (ii) MOLECULE TYPE: cDNA

25 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

TCGACCCACG CGTCCG

16

(2) INFORMATION FOR SEQ ID NO:8:

30 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
35 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

40

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

CGGACGCGTG GG

12

45 (2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

50 (A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

55

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

TGTAAAACGA CGGCCAGT

18

5 (2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 18 base pairs

(B) TYPE: nucleic acid

10 (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

15

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

20 CAGGAAACAG CTATGACC

18

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

25 (A) LENGTH: 20 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

30 (ii) MOLECULE TYPE: cDNA

35 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

CAATTAACCC TACTAAAGG

20

40 (2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 32 base pairs

(B) TYPE: nucleic acid

45 (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

50

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

55 GACTAGTCAG ACCTGGACAG CAGTATGCCT TC

32

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 45 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

10

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

15

ATAAGAATGC GGCCGCTAAA CTATGGGTAC TTTAGGGCTG TGTTT

45

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:

- 20 (A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
25 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

30

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

GCCTCTAGAA AGAGCTGGGA C

21

35 (2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:

- 40 (A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

45

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

50 CGCCGTGTTC CATTTATGAG C

21

WHAT IS CLAIMED IS:

1. An isolated nucleic acid encoding a
5 polypeptide comprising at least one of the biological
activities of AGP-1 wherein the nucleic acid is selected
from the group consisting of:
 - a) the nucleic acids shown in Figure 1 (SEQ ID
NO: 1) or Figure 2 (SEQ ID NO: 3);
 - 10 b) nucleic acids which hybridize to the
polypeptide coding regions of the nucleic acids shown in
Figure 1 (SEQ ID NO: 1) or Figure 2 (SEQ ID NO: 3) and
remain hybridized to the nucleic acids under high
stringency conditions; and
 - 15 c) nucleic acids which are degenerate to the
nucleic acids of (a) or (b).
2. The nucleic acid of Claim 1 which is cDNA,
genomic DNA, synthetic DNA or RNA.
20
3. A polypeptide encoded by the nucleic acid
of Claim 1.
4. The nucleic acid of Claim 1 including one
25 or more codons preferred for Escherichia coli
expression.
5. The nucleic acid of Claim 1 having a
detectable label attached thereto.
30
6. The nucleic acid of Claim 1 comprising the
polypeptide-coding region of Figure 2 (SEQ ID NO: 3).
7. A nucleic acid encoding a polypeptide
35 having the amino acid sequence of SEQ. ID. NO. 2 or SEQ.
ID. NO. 4.

8. An expression vector comprising the nucleic acid of Claim 1.

5 9. The expression vector of Claim 8 wherein the nucleic acid comprises the polypeptide-encoding region as shown in Figure 1 (SEQ ID NO: 1) or Figure 2 (SEQ ID NO: 3).

10 10. A host cell transformed or transfected with the expression vector of Claim 8.

 11. The host cell of Claim 10 which is a eucaryotic or procaryotic cell.

15 12. The host cell of Claim 11 which is Escherichia coli.

 13. A process for the production of AGP-1
20 comprising:
 growing under suitable nutrient
 conditions host cells transformed or transfected with
 the nucleic acid of Claim 1; and
 isolating the polypeptide product of the
25 expression of the nucleic acid.

 14. A polypeptide produced by the process of Claim 13.

30 15. A purified and isolated AGP-1 polypeptide.

 16. The polypeptide of Claim 15 which is mammalian AGP-1.

35 17. The polypeptide of Claim 15 having the amino acid sequence as shown in Figure 2 (SEQ ID NO: 3).

18. The polypeptide of Claim 17 which has been covalently modified with a water-soluble polymer.

5 19. The polypeptide of Claim 18 wherein the polymer is polyethylene glycol.

 20. An antibody or fragment thereof which specifically binds AGP-1.

10

 21. The antibody of Claim 20 which is a monoclonal antibody.

 22. A method for detecting the presence of AGP-1 in a biological sample comprising:

15 incubating the sample with the antibody of Claim 20 under conditions that allow binding of the antibody to AGP-1; and
 detecting the bound antibody.

20

 23. A method to assess the ability of a candidate compound to bind AGP-1 comprising:

 incubating AGP-1 with the candidate compound under conditions that allow binding; and
25 measuring the bound compound.

 24. The method of Claim 23 wherein the compound is an antagonist of AGP-1.

30 25. A method of regulating expression of AGP-1 in an animal comprising administering to the animal a nucleic acid complementary to the nucleic acids as shown in Figure 1 (SEQ ID NO: 1) or Figure 2 (SEQ ID NO: 3).

35 26. A pharmaceutical composition comprising a therapeutically effective amount of AGP-1 in a

pharmaceutically acceptable carrier, adjuvant,
solubilizer, stabilizer and/or anti-oxidant.

27. The composition of Claim 26 wherein AGP-1
5 is human AGP-1.

28. A method of treating an inflammatory
disorder comprising administering a therapeutically
effective amount of an AGP-1 antagonist.
10

29. The method of Claim 28 further comprising
administering a therapeutically effective amount of an
anti-inflammatory agent selected from the group
consisting of a corticosteroid, a non-steroidal
15 anti-inflammatory agent, and a cyclosporin.

30. A method of treating a hematopoietic
disorder comprising administering a therapeutically
effective amount of AGP-1.
20

31. The method of Claim 30 further comprising
administering a therapeutically effective amount of a
hematopoietic factor selected from the group consisting
of EPO, G-CSF, MGDF, GM-CSF, SCF, IL-3 and IL-6.
25

32. A method of treating a bone disorder
comprising administering a therapeutically effective
amount of an AGP-1 antagonist.

33. The method of Claim 31 further comprising
administering a therapeutically effective amount of a
bone growth factor selected from the group consisting
of: bone morphogenic factors BMP-1 to BMP-12, TGF- β
family members, IL-1 inhibitors, TNF α inhibitors,
35 parathyroid hormone, E series prostaglandins,
bisphosphonates and bone-enhancing minerals.

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FIGURE 1

GTTCATAGAT GGGTTAGATC TCAGAGCGCT GGATCTAGGC TTTCCAGCAC CATCAGGGCG	60
AGCTCTCCTA GCTGGAGGGT TTCTGTGCAC TACGTCTCG TCACCTTCCT GACTTGCTTA	120
GTTTCACTTT TGGTCTCAAC AGTAAAAAGA AACTGCATGG GCACTCCGCC TTCTAACTGT	180
GACCTTCTCA GGCCTGCTG CTGGGCTGCA AGTCTGCATT GGGAAATCAG ACCTGGACAG	240
CAGT ATG CCT TCC TCA GGG GCC CTG AAG GAC CTC AGC TTC AGT CAG CAC Met Pro Ser Ser Gly Ala Leu Lys Asp Leu Ser Phe Ser Gln His 1 5 10 15	289
TTC AGG ATG ATG GTG ATT TGC ATA GTG CTC CTG CAG GTG CTC CTG CAG Phe Arg Met Met Val Ile Cys Ile Val Leu Leu Gln Val Leu Leu Gln 20 25 30	337
GCT GTG TCT GTG GCT GTG ACT TAC ATG TAC TTC ACC AAC GAG ATG AAG Ala Val Ser Val Ala Val Thr Tyr Met Tyr Phe Thr Asn Glu Met Lys 35 40 45	385
CAG CTG CAG GAC AAT TAC TCC AAA ATT GGA CTA GCT TGC TTC TCA AAG Gln Leu Gln Asp Asn Tyr Ser Lys Ile Gly Leu Ala Cys Phe Ser Lys 50 55 60	433
ACG GAT GAG GAT TTC TGG GAC TCC ACT GAT GGA GAG ATC TTG AAC AGA Thr Asp Glu Asp Phe Trp Asp Ser Thr Asp Gly Glu Ile Leu Asn Arg 65 70 75	481
CCC TGC TTG CAG GTT AAG AGG CAA CTG TAT CAG CTC ATT GAA GAG GTG Pro Cys Leu Gln Val Lys Arg Gln Leu Tyr Gln Leu Ile Glu Glu Val 80 85 90 95	529
ACT TTG AGA ACC TTT CAG GAC ACC ATT TCT ACA GTT CCA GAA AAG CAG Thr Leu Arg Thr Phe Gln Asp Thr Ile Ser Thr Val Pro Glu Lys Gln 100 105 110	577
CTA AGT ACT CCT CCC TTG CCC AGA GGT GGA AGA CCT CAG AAA GTG GCA Leu Ser Thr Pro Pro Leu Pro Arg Gly Gly Arg Pro Gln Lys Val Ala 115 120 125	625
GCT CAC ATT ACT GGG ATC ACT CGG AGA AGC AAC TCA GCT TTA ATT CCA Ala His Ile Thr Gly Ile Thr Arg Arg Ser Asn Ser Ala Leu Ile Pro 130 135 140	673
ATC TCC AAG GAT GGA AAG ACC TTA GGC CAG AAG ATT GAA TCC TGG GAG Ile Ser Lys Asp Gly Lys Thr Leu Gly Gln Lys Ile Glu Ser Trp Glu 145 150 155	721
TCC TCT CGG AAA GGG CAT TCA TTT CTC AAC CAC GTG CTC TTT AGG AAT Ser Ser Arg Lys Gly His Ser Phe Leu Asn His Val Leu Phe Arg Asn 160 165 170 175	769
GGA GAG CTG GTC ATC GAG CAG GAG GGC CTG TAT TAC ATC TAT TCC CAA Gly Glu Leu Val Ile Glu Gln Glu Gly Leu Tyr Tyr Ile Tyr Ser Gln 180 185 190	817

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FIGURE 1 (cont.)

ACA TAC TTC CGA TTT CAG GAA GCT GAA GAC GCT TCC AAG ATG GTC TCA	865
Thr Tyr Phe Arg Phe Gln Glu Ala Glu Asp Ala Ser Lys Met Val Ser	
195 200 205	
AAG GAC AAG GTG AGA ACC AAA CAG CTG GTG CAG TAC ATC TAC AAG TAC	913
Lys Asp Lys Val Arg Thr Lys Gln Leu Val Gln Tyr Ile Tyr Lys Tyr	
210 215 220	
ACC AGC TAT CCG GAT CCC ATA GTG CTC ATG AAG AGC GCC AGA AAC AGC	961
Thr Ser Tyr Pro Asp Pro Ile Val Leu Met Lys Ser Ala Arg Asn Ser	
225 230 235	
TGT TGG TCC AGA GAT GCC GAG TAC GGA CTG TAC TCC ATC TAT CAG GGA	1009
Cys Trp Ser Arg Asp Ala Glu Tyr Gly Leu Tyr Ser Ile Tyr Gln Gly	
240 245 250 255	
GGA TTG TTC GAG CTA AAA AAA AAT GAC AGG ATT TTT GTT TCT GTG ACA	1057
Gly Leu Phe Glu Leu Lys Lys Asn Asp Arg Ile Phe Val Ser Val Thr	
260 265 270	
AAT GAA CAT TTG ATG GAC CTG GAT CAA GAA GCC AGC TTC TTT GGA GCC	1105
Asn Glu His Leu Met Asp Leu Asp Gln Glu Ala Ser Phe Phe Gly Ala	
275 280 285	
TTT TTA ATT AAC TAA ATGACCAGTA AAGATCAAAC ACAGCCCTAA AGTACCCAGT	1160
Phe Leu Ile Asn *	
290	
AATCTTCTAG GTTGAAGGCA TGCCTGGAAA GCGACTGAAC TGGTTAGGAT ATGGCCTGGC	1220
TGTAGAAACC TCAGGACAGA TGTGACAGAA AGGCAGCTGG AACTCAGCAG CGACAGGCCA	1280
ACAGTCCAGC CACAGACACT TTCGGTGTTT CATCGAGAGA CTTGCTTTCT TTCCGCAAAA	1340
TGAGATCACT GTAGCCTTTC AATGATCTAC CTGGTATCAG TTTGCAGAGA TCTAGAAGAC	1400
GTCCAGTTTC TAAATATTTA TGCAACAATT GACAATTTTC ACCTTTGTTA TCTGGTCCAG	1460
GGGTGTAAAG CCAAGTGCTC ACAGGCTGTG TGCAGACCAG GATAGCTATG AATGCAGGTC	1520
AGCATAAAAA TCACAGAATA TCTCACCTAC CAAATCAGAG TGGGTGTGCC CCTGTGTGTA	1580
TATGCGTGTC TGTGTGTGTG TGCATGTATG TGTGTGTGTG TGTGACTGTT CTTTATGGTA	1640
ACTGGTTATG TTTTCTCAA GTGAAAAACA TAACTCTATA CATGATAACA TAATATCCCA	1700
TCATCAGTGG AACCTTGCCC AAAGAATGTA TGAAATCTCC AGGCAATGAA TGAGGGCAGC	1760
CCAAGAAAGA GGCCCGCAGA GCCATACCAC AGGGCTGCCC CACCCTGCTG GAGCTCAGAT	1820
CCTGCCACTG CTGCAGGCCC TGGGTACCAG GTGTAGAGTT GGAGGAGGTC TTGCCTGTGG	1880
GTCTAGGTCT TTGGTGCCTA CCTCCTTGAT ATGGCCCCAG TCCTCCTTTG CTTGTTTGCT	1940
AGTTTTATCA TGTTTCCCAG GCCGGCCTCA AGTCCAATAT GTAGTCAAGA GTGATCTCTA	2000

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FIGURE 1 (cont.)

ACTGTGCAAC CTCCTGCCTC CAAGATCTGC TGAGATTATA GGCATGTGCC CCCCTGTCTG	2060
ATTTGTGTAG AGCCAGGCTT CTTGTACATG TGACAACCAT GCCACCCTCA GCTCTGTCCC	2120
AGCTCCATTT CTTCTTTTCT GAATGCAAGC ATTTACTTTG TGTCCCTATA TTCTAGAATG	2180
TGCAACAGTG AAGAATTTGC TCTGACTTTC AGGATAAAGT TTGAACTAGG TTCACCATGC	2240
TTGCTTTGTC CAGATTGCGA CTGTCACCCA GTCCTCTGGC TCTTCCATCT GTCTGTCCAC	2300
TCCACCTACC AAGATGTTGA ACACTTGTTT TTTTAAAGAT GTTGGTGCCT GGAGTTTCAT	2360
TAGAGTAACA CAAAACCTAAC TAAACCAAAA CAACTCCAAA GGAGCCCATATA TGTGTTTTAA	2420
TGAAACATTT TTTAAGCCTA TTGGGGGCCT GAAGAGATTG CTCAGAGGAA AACAGCACTT	2480
CCAGAGGACC CAGGTTCAAT TCTCATCGCT GATGTGATAG TTAACAGCTG TAACTTCAGT	2540
TCCAAGGGGT CTGACTTTTCT GCCCTTTGCT TGCAATGCAT GTATGTGATA CACAGACATA	2600
CATTCTGACA AAATATATCC ATACACAAAA GTATTTTTTT AAAAGCTTAT TTGAATGTAA	2660
GAGTATGGCT AGCTGTCACT TCTGATACCC CTTCTTATTT TTTTATGACT CAAGCCCTTA	2720
TAAACTAGCA AATAGAAGTC ACAGCTACCA CTTGAATATA AGCACTTGAA TACCTCCTCT	2780
CACTAGAATA CAACATAGCT TAATAGTAAA AATCTTGCCT TAGTAAAGTA CTTGCATGTC	2840
ATGTCTACAT GAACCAAATG AATGTATTAA TTAATAATAG ACATAATGAT CACATCGGAA	2900
AGGCTGTGAG AAATAATGGA GAACATTTGA AAGCTCAAGA TGGAAGGGAA AGGCACTTGT	2960
CAAAAATCTT GACAACCTGA ATTTGACCTT TGGCAGGGCT GAAAACATAA CCCAGGGTCT	3020
TACTCCCAGT AGGCATGAAC TCCCCCCT	3048

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FIGURE 2

GGCTGACTTA CAGCAGTCAG ACTCTGACAG GATC ATG GCT ATG ATG GAG GTC	52
Met Ala Met Met Glu Val	
1 5	
CAG GGG GGA CCC AGC CTG GGA CAG ACC TGC GTG CTG ATC GTG ATC TTC	100
Gln Gly Gly Pro Ser Leu Gly Gln Thr Cys Val Leu Ile Val Ile Phe	
10 15 20	
ACA GTG CTC CTG CAG TCT CTC TGT GTG GCT GTA ACT TAC GTG TAC TTT	148
Thr Val Leu Leu Gln Ser Leu Cys Val Ala Val Thr Tyr Val Tyr Phe	
25 30 35	
ACC AAC GAG CTG AAG CAG ATG CAG GAC AAG TAC TCC AAA AGT GGC ATT	196
Thr Asn Glu Leu Lys Gln Met Gln Asp Lys Tyr Ser Lys Ser Gly Ile	
40 45 50	
GCT TGT TTC TTA AAA GAA GAT GAC AGT TAT TGG GAC CCC AAT GAC GAA	244
Ala Cys Phe Leu Lys Glu Asp Asp Ser Tyr Trp Asp Pro Asn Asp Glu	
55 60 65 70	
GAG AGT ATG AAC AGC CCC TGC TGG CAA GTC AAG TGG CAA CTC CGT CAG	292
Glu Ser Met Asn Ser Pro Cys Trp Gln Val Lys Trp Gln Leu Arg Gln	
75 80 85	
CTC GTT AGA AAG ATG ATT TTG AGA ACC TCT GAG GAA ACC ATT TCT ACA	340
Leu Val Arg Lys Met Ile Leu Arg Thr Ser Glu Glu Thr Ile Ser Thr	
90 95 100	
GTT CAA GAA AAG CAA CAA AAT ATT TCT CCC CTA GTG AGA GAA AGA GGT	388
Val Gln Glu Lys Gln Gln Asn Ile Ser Pro Leu Val Arg Glu Arg Gly	
105 110 115	
CCT CAG AGA GTA GCA GCT CAC ATA ACT GGG ACC AGA GGA AGA AGC AAC	436
Pro Gln Arg Val Ala Ala His Ile Thr Gly Thr Arg Gly Arg Ser Asn	
120 125 130	
ACA TTG TCT TCT CCA AAC TCC AAG AAT GAA AAG GCT CTG GGC CGC AAA	484
Thr Leu Ser Ser Pro Asn Ser Lys Asn Glu Lys Ala Leu Gly Arg Lys	
135 140 145 150	
ATA AAC TCC TGG GAA TCA TCA AGG AGT GGG CAT TCA TTC CTG AGC AAC	532
Ile Asn Ser Trp Glu Ser Ser Arg Ser Gly His Ser Phe Leu Ser Asn	
155 160 165	
TTG CAC TTG AGG AAT GGT GAA CTG GTC ATC CAT GAA AAA GGG TTT TAC	580
Leu His Leu Arg Asn Gly Glu Leu Val Ile His Glu Lys Gly Phe Tyr	
170 175 180	
TAC ATC TAT TCC CAA ACA TAC TTT CGA TTT CAG GAG GAA ATA AAA GAA	628
Tyr Ile Tyr Ser Gln Thr Tyr Phe Arg Phe Gln Glu Ile Lys Glu	
185 190 195	
AAC ACA AAG AAC GAC AAA CAA ATG GTC CAA TAT ATT TAC AAA TAC ACA	676
Asn Thr Lys Asn Asp Lys Gln Met Val Gln Tyr Ile Tyr Lys Tyr Thr	
200 205 210	

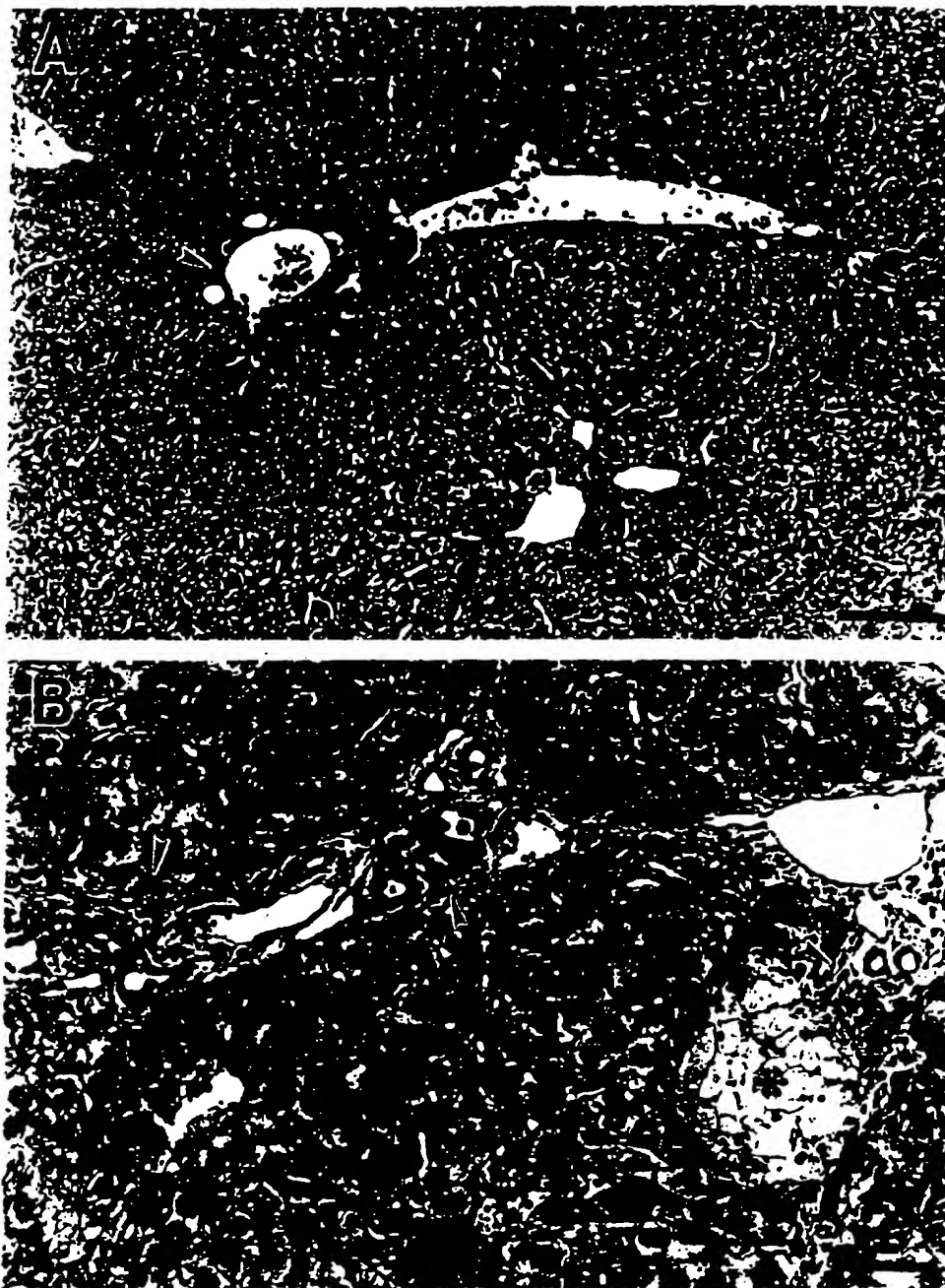
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FIGURE 2 (cont.)

AGT TAT CCT GAC CCT ATA TTG TTG ATG AAA AGT GCT AGA AAT AGT TGT Ser Tyr Pro Asp Pro Ile Leu Leu Met Lys Ser Ala Arg Asn Ser Cys 215 220 225 230	724
TGG TCT AAA GAT GCA GAA TAT GGA CTC TAT TCC ATC TAT CAA GGG GGA Trp Ser Lys Asp Ala Glu Tyr Gly Leu Tyr Ser Ile Tyr Gln Gly Gly 235 240 245	772
ATA TTT GAG CTT AAG GAA AAT GAC AGA ATT TTT GTT TCT GTA ACA AAT Ile Phe Glu Leu Lys Glu Asn Asp Arg Ile Phe Val Ser Val Thr Asn 250 255 260	820
GAG CAC TTG ATA GAC ATG GAC CAT GAA GCC AGT TTT TTC GGG GCC TTT Glu His Leu Ile Asp Met Asp His Glu Ala Ser Phe Phe Gly Ala Phe 265 270 275	868
TTA GTT GGC TAA CTGACCTGGA AAGAAAAAGC AATAACCTCA AAGTGA CTAT Leu Val Gly * 280	920
TCAGTTTTCA GGATGATACA CTATGAAGAT GTTTCAAAAA ATCTGACCAA AACAAACAAA	980
CAGAAAACAG AAAACAAAAA AACCTCTATG CAATCTGAGT AGAGCAGCCA CAACCAAAAT	1040
TGTATACAAC ACACCATGTA	1060

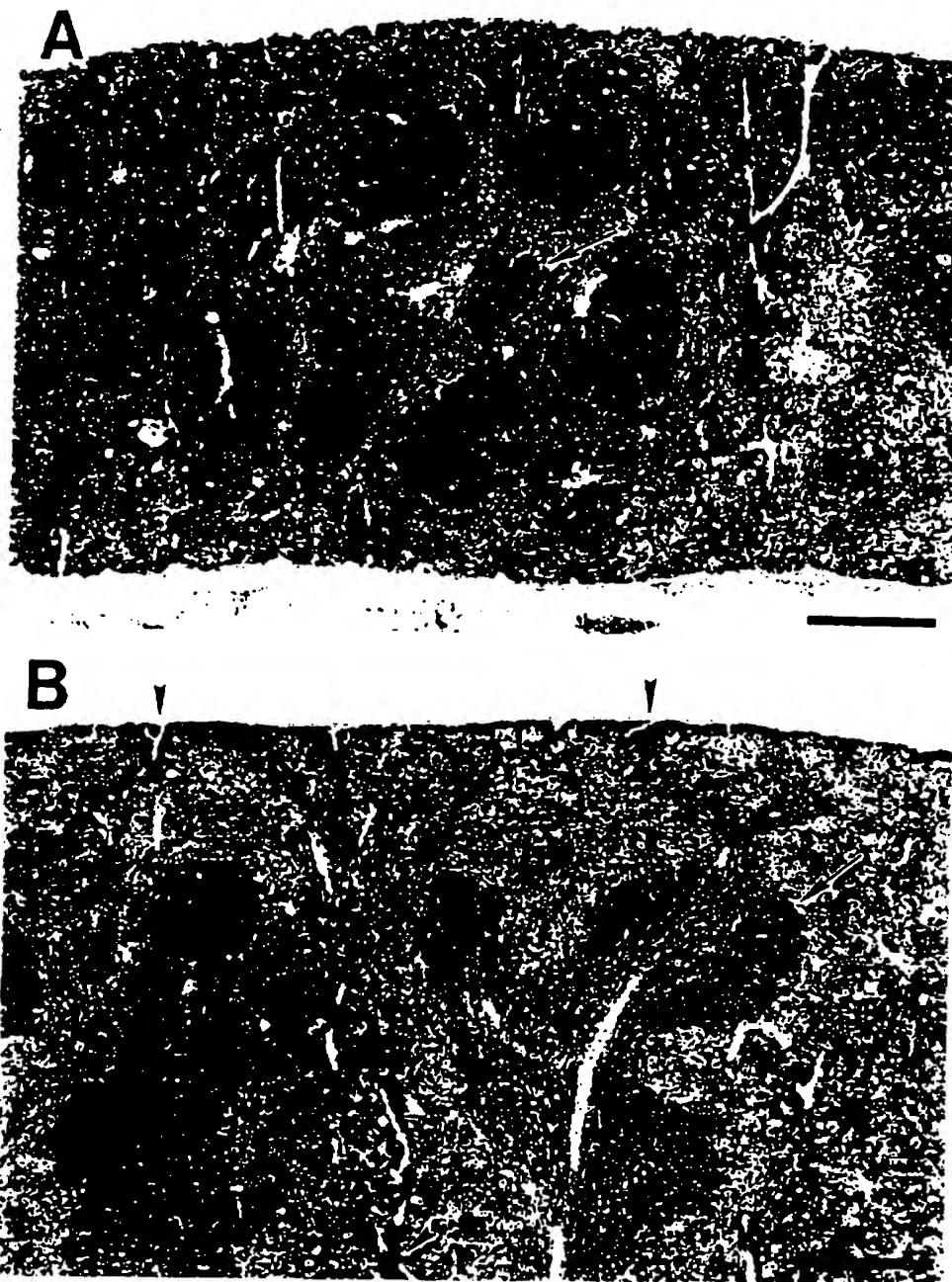
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FIGURE 3



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FIGURE 4



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FIGURE 5

